

# Implications of $\beta$ -glucanase and pentosanase enzymes in low-energy low-protein barley and wheat based broiler diets

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**ABSTRACT:** This study was conducted to test the effects of a commercial enzyme (with  $\beta$ -glucanase and pentosanase activities) supplemented into low-protein low-energy barley and wheat based broiler diets on broiler performance. The enzyme was added at 500 g/ton into broiler grower and finisher diets consisting of mainly wheat at 76%, 85% or barley 67%, 75%, respectively. Four dietary treatments were wheat, wheat + enzyme, barley, barley + enzyme. Each treatment had six replications. This experiment was planned according to a completely randomised design by placing ten 14-day-old mixed male and female chicks into one experimental cage unit with wire floor. Cobb broiler chicks were used in this study. Experimental grower and finisher diets were fed to chicks between 14–28 and 28–42 days of age, respectively. One-day-old chicks were fed a standard starter diet (23% protein; 12.77 MJ ME/kg) according to NRC (1994) recommendations. Grower diet and finisher diets were formulated to be 10% lower than NRC (1994) with respect to the protein and metabolisable energy content. Body weight, average weight gain (14–42 days period), feed intake and feed efficiency ratio were measured at 42 days of age. The results of this study demonstrated that the enzyme with  $\beta$ -glucanase and pentosanase activities supplemented into barley-based broiler diets significantly ( $P < 0.05$ ) improved body weight by 10%, from 1 779 to 1 958 g, and gain by 12%, from 1 485 to 1 657 g, respectively. However, when the same enzyme was supplemented into wheat-based diets, no improvement ( $P < 0.05$ ) was obtained in body weight and feed efficiency, being 1 723 and 1 677 g and 1 973 and 1 957, respectively for wheat and wheat + enzyme groups. The feed efficiency ratio was also significantly ( $P < 0.05$ ) improved in barley-based diet from 1.898 to 1.845 by enzyme addition during the 14–42 days experimental period.

**Keywords:**  $\beta$ -glucanase; pentosanase; enzymes; barley; wheat; broiler diets

Wheat and barley can be more cost effective feed ingredients compared to maize as the major grain in broiler diets especially during the harvest season in various regions of the world. However, wheat and barley contain considerably higher levels of anti-nutritional factors consisting mainly of water-soluble and nonsoluble nonstarch polysaccharides (NSP) compared to maize.

The most important NSP fraction in wheat and barley has been reported to be  $\beta$ -glucans and pentosans (Hesselman and Aman, 1986; Annison, 1991; Jeroch *et al.*, 1992; Bedford, 2000). These substances have been found to adversely effect nutrient digestion, absorption and gut microflora. Especially the water-soluble NSP content found in barley and wheat was reported to increase digesta viscosity

in the chicken gut (Hesselman and Aman, 1986; Bedford *et al.*, 1991; Choct and Annison, 1992). The implications of the physicochemical and anti-nutritive properties of NSP were documented very well in the review study conducted by Smiths and Annison (1996).  $\beta$ -glucan solubilisation results in a viscous condition of the digesta that interferes with nutrient assimilation within the chick intestine (Edney *et al.*, 1989). Insoluble  $\beta$ -glucans may impair starch digestibility by impeding the accessibility of starch granules to the amylolytic activity within the intestine (Hesselman and Aman, 1986). Enzyme addition to a barley-based diet improves performance of broiler chicks (Pettersson *et al.*, 1990), but improvements are less pronounced in older broilers.

High levels of wheat inclusion into wheat-based broiler diets also had unfavourable effects on broiler performance. The anti-nutritive activity of pentosans in wheat is also related to the increase in digesta viscosity (Choct and Annison, 1990, 1992). The increased viscosity reduces intestinal digestion and absorption because of the interaction between pentosans and endogenous enzymes that promotes the formation of a complex that reduces the activity of these enzymes (Nahas and Lefrançois, 2001). Therefore, microbial enzymes have been found to be beneficial in terms of nutrient utilisation when supplemented into barley or wheat based broiler diets (Edney *et al.*, 1989; Petterson *et al.*, 1990; Nahas and Lefrençois, 2001).

On the other hand, to balance the energy need of broiler diets can be a problem where maize production or other energy sources such as full-fat soya and fats are scarce. Under these conditions broiler diets with 12.56–13.81 MJ/kg metabolisable energy levels are difficult to balance. However, it should not be any problem to prepare broiler diets based on wheat and barley at 10% lower energy content compared to NRC (1994) recommendations ranging from 11.72 to 12.56 MJ/kg.

Therefore, this study was carried out mainly to test the effects of an enzyme with  $\beta$ -glucanase and pentosanase activities in low-energy low-protein broiler diets using wheat and barley as the main cereal grain.

## MATERIAL AND METHODS

### Animals, diets and husbandry

Male and female as hatched 240 Cobb breed chicks were used in this experiment. Chicks hatched on the same day were brooded on wood shavings floor and fed a pre-experimental starter diet (23% protein; 12.77 MJ/kg metabolisable energy) mainly consisting of maize, wheat and soybean until 14 days of age. In the experimental room lighting was 23-hour light and 1 hour dark. Feed and water were provided *ad libitum*.

At 14 days of age birds were individually weighed and randomly allocated to wire floor battery type experimental cages so as to place 10 birds at closer weight average into each cage. Thereafter, the birds were given experimental dietary grower and finisher feeds. Dietary treatments consisted of (1) wheat, (2) wheat + enzyme, (3) barley and (4)

barley + enzyme based diets. The chemical composition of test diets is illustrated in Tables 1 and 2. Metabolisable energy levels of the test diets were restricted and kept lower than NRC (1994) recommendations, being 11.90 MJ/kg and 12.00 MJ/kg in grower and finisher diets, respectively. Dietary protein levels of the test grower and finisher diets were also relatively lower than NRC (1994) being 19% and 16%, respectively. A commercial NSP enzyme Endofeed (GNC BIOFERM INC.) with 1 000 IU/g  $\beta$ -glucanase and 1 500 IU/g pentosanase activity was supplemented into the related wheat and barley based diets at 500 g/ton.

### Experimental design and measurements

This trial was planned according to a completely randomised design. Thus, each of the dietary treatment had 6 replications in which 10 birds were assigned. Experimental birds freely accessed to test diets and water from feed troughs and nipple drinkers. Experimental cage units were kept in the environmentally controlled room. Lighting was controlled by providing 23-hour light and 1-hour dark from 14 to 42 days of age. The experiment was terminated at 42 days of age.

At the termination of the experiment birds were weighed by the cage unit and feed consumption was determined. Body weight, weight gain, feed consumption and feed efficiency (feed/gain ratio) were measured. The collected data was subjected to analysis of variance and the differences between means were analysed according to Duncan's Multiple Range Test. The results were presented as means with the standard deviation for each mean. Type I error controlled at  $P = 0.05$  for evaluating treatment effects.

## RESULTS

The results of this trial are shown in Table 3. There were statistically significant differences between dietary treatments in terms of body weight, gain and feed consumption and feed efficiency. The type of cereal did not significantly ( $P > 0.05$ ) affect bird performance with respect to body weight. Enzyme supplementation to wheat-based diet did not affect body weight whereas it significantly affected body weight in barley-based diet ( $P < 0.05$ ). In the group fed barley, body weight

Table 1. Experimental diets (broiler grower)

Ingredients (g/kg diet)	Wheat	Barley	Wheat + enzyme	Barley + enzyme
Barley	–	670.6	–	671.0
Wheat	760.7	–	760.2	–
Soybean meal	166.0	216.7	166.0	216.7
Fish meal	30.0	28.2	30.0	28.2
Vegetable oil	9.4	53.5	9.4	53.5
Dicalcium phosphate	15.0	14.7	15.0	14.7
Limestone	11.5	9.6	11.5	9.6
Vitamin + Mineral <sup>1</sup>	2.5	2.5	2.5	2.5
Common salt	2.5	2.5	2.5	2.5
L-Lysine	0.7	–	0.7	–
DL-Methionine	1.7	1.7	1.7	1.7
Enzyme	–	–	0.5	0.5
Total	1 000.0	1 000.0	1 000.0	1 000.0
Calculated values (g/kg diet)				
ME, MJ/kg	11.90	11.90	11.90	11.90
Crude protein	19.00	19.00	19.00	19.00
Crude fibre	2.62	7.41	2.62	7.41
Ether extract	2.06	4.49	2.06	4.49
Lysine	1.00	1.00	1.00	1.00
Methionine	0.48	0.48	0.48	0.48
Met + Cys	0.79	0.79	0.79	0.79
Calcium	0.90	0.90	0.90	0.90
Available phosphorus	0.46	0.46	0.46	0.46
Sodium	0.18	0.18	0.18	0.18

<sup>1</sup>Vitamin-mineral (per kg premix): 12 500 IU vit A, 2 500 IU vit D<sub>3</sub>, 30 mg vit E, 4.5 mg vit. K, 1.8 mg vit. B<sub>1</sub>, 6 mg vit B<sub>2</sub>, 2.4 mg vit B<sub>6</sub>, 0.02 mg vit B<sub>12</sub>, 10 mg niacin, 12 mg Ca-D-pantothenate, 0.6 mg folic acid, 0.06 mg biotin, 1 600 mg Ca, 1 300 mg P, 0.4 mg Mg, 3.2 mg Zn, 3.0 mg Mn, 8 mg Cu, 0.4 mg I, 0.1 mg Co, 0.1 mg Se, 500 mg NaCl, 300 mg NaHCO<sub>3</sub>

increased from 1 779 to 1 958 g by the enzyme supplementation. This increase in body weight was significant ( $P < 0.05$ ) and about 10% higher than that of the non-enzyme supplemented group. However, in wheat group the enzyme supplementation numerically lowered body weight. Enzyme supplementation affected weight gain in a similar manner like the body weight. Moreover, enzyme supplementation significantly affected gain only in the group fed barley, but not in the group fed wheat-based diet.

Feed consumption was also significantly affected by enzyme addition. In this parameter, the same manner was observed concerning the effect of enzyme on wheat and barley based diets. Although the feed intake of wheat and barley based dietary treatment groups was almost similar being 2 814 g and 2 817 g for wheat and barley respectively, in enzyme-supplemented corresponding diets the enzyme supplementation significantly increased feed intake in barley group while it significantly decreased feed intake in the group fed wheat-based

Table 2. Experimental diets (broiler finisher)

Ingredients (g/kg diet)	Wheat	Barley	Wheat + enzyme	Barley + enzyme
Barley	–	745.7	–	745.2
Wheat	847.5	–	847.0	–
Soybean meal	106.5	163.8	106.5	163.8
Fish meal	9.8	–	9.8	–
Vegetable oil	–	54.8	–	54.8
Dicalcium phosphate	14.6	15.8	14.6	15.8
Limestone	12.0	12.3	12.0	12.3
Vitamin + Mineral <sup>1</sup>	2.5	2.5	2.5	2.5
Common salt	2.5	2.7	2.5	2.7
L-Lysine	2.4	0.4	2.4	0.4
DL-Methionine	2.2	2.0	2.2	2.0
Enzyme	–	–	0.5	0.5
Total	1 000.0	1 000.0	1 000.0	1 000.0
Calculated values (g/kg diet)				
ME, MJ/kg	12.00	12.00	12.00	12.00
Crude protein	16.00	16.00	16.00	16.00
Crude fibre	2.80	5.27	2.80	5.27
Ether extract	1.89	7.32	1.89	7.32
Lysine	0.85	0.85	0.85	0.85
Methionine	0.46	0.46	0.46	0.46
Met + Cys	0.75	0.77	0.75	0.77
Calcium	0.90	0.86	0.90	0.86
Available phosphorus	0.43	0.43	0.43	0.43
Sodium	0.18	0.18	0.18	0.18

<sup>1</sup>Vitamin-mineral (per kg premix): 12 500 IU vit A, 2 500 IU vit D<sub>3</sub>, 30 mg vit E, 4.5 mg vit. K, 1.8 mg vit. B<sub>1</sub>, 6 mg vit B<sub>2</sub>, 2.4 mg vit B<sub>6</sub>, 0.02 mg vit B<sub>12</sub>, 10 mg niacin, 12 mg Ca-D-pantothenate, 0.6 mg folic acid, 0.06 mg biotin, 1 600 mg Ca, 1 300 mg P, 0.4 mg Mg, 3.2 mg Zn, 3.0 mg Mn, 8 mg Cu, 0.4 mg I, 0.1 mg Co, 0.1 mg Se, 500 mg NaCl, 300 mg NaHCO<sub>3</sub>

Table 3. Effects of enzyme supplementation into wheat and barley based broiler grower and finisher diets (14–42 days of age)

	Wheat	Wheat + enzyme	Barley	Barley + enzyme
Body weight (g)	1 723 ± 46 <sup>ab</sup>	1 677 ± 61 <sup>a</sup>	1 779 ± 42 <sup>b</sup>	1 958 ± 37 <sup>c</sup>
Gain (g)	1 426 ± 46 <sup>ab</sup>	1 384 ± 65 <sup>a</sup>	1 485 ± 44 <sup>b</sup>	1 657 ± 35 <sup>c</sup>
Feed intake (g)	2 814 ± 75 <sup>b</sup>	2 705 ± 90 <sup>a</sup>	2 817 ± 47 <sup>b</sup>	3 056 ± 55 <sup>c</sup>
Feed/Gain (kg/kg)	1.973 ± 0.020 <sup>b</sup>	1.957 ± 0.076 <sup>b</sup>	1.898 ± 0.067 <sup>ab</sup>	1.845 ± 0.044 <sup>a</sup>

<sup>a–c</sup>means in each row differ significantly ( $P < 0.05$ )

diet. The enzyme positively affected feed intake only in barley-based diet.

Feed efficiency was significantly ( $P < 0.05$ ) improved by the enzyme addition in the group fed barley being the lowest with the value 1.845, whereas it was 1.898 in the non-enzyme supplemented barley-based diet. In the wheat-based group enzyme addition did not significantly improve feed efficiency ( $P < 0.05$ ).

## DISCUSSION

The aim of this study was to examine the effect of an NSP enzyme supplementation into low-energy low-protein wheat and barley based broiler diets on broiler performance.

The results of this experiment indicated that the enzyme significantly improved broiler live performance only when it was added into barley-based diet. The same enzyme supplementation, however, did not positively affect broiler performance when it was added into wheat-based diets although it contains pentosanase (1 500 IU/g) and  $\beta$ -glucanase (1 000 IU/g) activities. Positive results observed with enzyme addition into barley-based diet were probably associated with reduction in digesta viscosity as previously reported by researchers (Petterson *et al.*, 1990; Choct and Annison, 1990). The increase in feed intake resulted in increased gain and body weight in the related barley fed group. Feed efficiency was also improved by enzyme addition into barley-based diets. As it was clearly demonstrated by many researchers (Rotter *et al.*, 1990; Bedford *et al.*, 1991; Choct and Annison, 1992), the  $\beta$ -glucan content of barley consisting of water-soluble and non-soluble fractions is responsible for increased gut viscosity. As a result, the digestion of nutrients with digestive enzymes, diffusion and convective transport of lipase, oils and bile salt micelles within the gastrointestinal content might be impaired. It was reported that these effects resulted in wet droppings, depressed growth and feed efficiency. In addition, NSP enzymes with particularly  $\beta$ -glucanase activity break this  $\beta$ -1.3,  $\beta$ -1.4 glycosidic bonds found in  $\beta$ -glucans of barley and alleviate the negative effects of high viscosity. Thus, overall digestion of nutrients, namely fats, proteins and starch are improved.

Although it was suggested (Newman and Newman, 1988; Dibner *et al.*, 1996) that enzyme addition into barley-based diets could be better

pronounced at younger age, since the chicken's digestive system undergoes changes and becomes more capable of efficiently digesting the nutrients, in the present experiment the improved nutrient utilisation by older birds in enzyme supplemented barley-based groups, between 14 to 42 days of age, seemed to be possible.

Pentosanase and  $\beta$ -glucanase activities of this enzyme did not seem to positively affect the pentosan and  $\beta$ -glucan content of wheat used in this trial. These results are in accordance with the findings reported by Brufau *et al.* (2001), Austin *et al.* (1999) and Moisonnier *et al.* (2001). Austin *et al.* (1999) indicated that AME of wheat was not correlated with the amount of total water-soluble NSP, soluble pentosans or  $\beta$ -glucans. Moisonnier *et al.* (2001) demonstrated that the low apparent digestibility of starch and lipid observed with wheat could not be attributed only to intestinal viscosity. The consequences of inefficiency of enzyme preparations observed in wheat might be caused by the presence of enzyme inhibitors. In the present study, the improved nutrient utilisation by older birds in barley-based groups, between 14 to 42 days of age, seemed to be possible in cereal grains as reported by Rouau and Surget (1998). These findings suggest that NSP enzymes suitable for wheat need not necessarily be always effective. In other words, the enzyme application to barley-based diets generally resulted in more consistent effects than to wheat. In barley the structural situation with respect to non-starch polysaccharides is a little bit different from wheat: the prominent cell wall component of endosperm and aleurone of barley is  $\beta$ -glucan, consisting of units of glucose joined by  $\beta$ -1.3 and  $\beta$ -1.4 bonds, makes up approximately 75% of this cell wall (Svihus *et al.*, 1997). On the other hand, in wheat the major cereal cell wall polysaccharides, pentosans, are more complex being composed of two sugars, arabinose and xylose, in a branched structure. Another comparative study (Jeroch and Dänicke, 1995) using broiler chicks with maize produced mostly negative results and the extent of the recorded growth depression depended mainly on the proportion of barley used. Villamide *et al.* (1997) detected a relationship between AME<sub>n</sub> and enzyme supplementation into barley-based diets. In their study Vranjes and Wenk (1995) also reported that an enzyme (with cellulase,  $\beta$ -glucanase and xylanase activities) supplementation into barley-based broiler diets significantly improved AME, fat and nitrogen utilisation.

Consequently, the results obtained under the conditions of this trial indicated that the enzyme (with  $\beta$ -glucanase and pentosanase activities) supplementation into low-energy low-protein barley based diets significantly improved gain and feed intake whereas it did not affect broiler performance when it was added into similar low-energy low-protein wheat based broiler grower and finisher diets.

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**ABSTRAKT****Důsledky podávání enzymů  $\beta$ -glukanázy a pentosanázy v nízkoenergetických nízkoproteinových krmných směsích pro brojlerky na bázi ječmene a pšenice**

Byly sledovány účinky komerčního enzymu (s  $\beta$ -glukanázou a pentosanázou) přidávaného do nízkoproteinových nízkoenergetických krmných směsí pro brojlerky na bázi ječmene a pšenice na užitkovost brojlerů. Enzym byl přidáván v dávce 500 g/tunu do krmných směsí grower a finisher brojlerů, které se skládaly ze 76% resp. 85% z pšenice nebo ze 67% resp. 75% z ječmene. Byly použity čtyři varianty směsí: pšenice, pšenice + enzym, ječmen, ječmen + enzym. Každá varianta měla šest opakování. Pokus byl uspořádán podle zcela randomizovaného schématu, kdy deset 14denních kohoutků a slepiček bylo umístěno do jedné pokusné klecové jednotky s roštovou podlahou. V tomto pokuse jsme použili kuřecí brojlerky hybridní kombinace Cobb. Pokusné krmné směsi grower a finisher byly kuřatům podávány mezi 14. až 28. resp. 28. až 42. dnem života. Jednodenní kuřata dostávala podle doporučení NRC (1994) standardní směs pro předvýkrm (23 % NL; 12,77 MJ ME/kg). Složení směsí grower a finisher bylo takové, aby byl obsah dusíkatých látek a metabolizovatelné energie o 10 % nižší než dle NRC (1994). Ve věku 42 dní byla zjišťována tělesná hmotnost kuřat, průměrný hmotnostní přírůstek (v období 14. až 42. dne života), příjem krmiva a konverze krmiva. Výsledky tohoto pokusu prokázaly, že přídavek enzymu s  $\beta$ -glukanázou a pentosanázou do krmných směsí pro brojlerky na bázi ječmene významně ( $P < 0,05$ ) zvýšil tělesnou hmotnost o 10 % (z 1 779 g na 1 958 g) a hmotnostní přírůstek o 12 % (z 1 485 na 1 657 g). Po přídavku stejného enzymu do směsí na bázi pšenice ke zvýšení ( $P < 0,05$ ) tělesné hmotnosti, ani ke zlepšení konverze krmiva nedošlo: hodnoty pro skupiny s pšenicí a pšenicí + enzym činily 1 723 g resp. 1 677 g a 1,973 resp. 1,957 kg/kg. Během pokusného období mezi 14. až 42. dnem života kuřat došlo rovněž vlivem přídavku enzymu k významnému ( $P < 0,05$ ) zlepšení konverze krmiva při podávání krmné směsi na bázi ječmene z 1,898 na 1,845 kg/kg.

**Klíčová slova:**  $\beta$ -glukanáza; pentosanáza; enzymy; ječmen; pšenice; krmné směsi pro brojlerky

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